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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/088,634	10/16/2002	Pal Maliga	RUT.00-0038US	7235

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PHILADELPHIA, PA 19103-2307

EXAMINER	
MEHTA, ASHWIN D	

ART UNIT	PAPER NUMBER
1638	

DATE MAILED: 04/04/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/088,634	MALIGA ET AL.	
	Examiner	Art Unit	
	Ashwin Mehta	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 October 2002*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-22* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-22* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *16 October 2002* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

HL

DETAILED ACTION

Specification

1. The paragraph on page 1, lines 9-14 of the specification should be deleted, as the information in that paragraph (priority information) also appears in the preliminary amendment to page 1 that was submitted on March 20, 2002.

2. The specification fails to comply with the sequence rules of 37 CFR 1.821-1.825. Nucleotide sequences appear on page 20, line 34- to page 21, line 2; page 21, lines 9, 10, 14-16; page 22, lines 5, 6, 15, 16, 20, 21, 27-29, 34; page 35, lines 33-35; and page 36, lines 11, 12, and 15-17, that must be referred to by their sequence identifiers.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. Claims 1-22 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

In claims 1 and 13: the recitation, "protein having excision activity" renders the claims indefinite. It is unclear exactly what kind of excision activity is being referred to. As the specification indicates that marker genes are removed from plastid genomes by using site-

specific recombinases, it is suggested that the recitation be replaced with --site-specific recombinase--.

Further in claims 1 and 13: the recitation, "thereby excising said predetermined target sequence" renders the claims indefinite. The claims do not clearly indicate what the predetermined target sequence is. The claims only indicate that the second DNA construct comprises excision sites, and that the excising activity acts on the excision sites. However, the claims do not state what the predetermined target sequence is. It is suggested that 1), step b) of the claims be amended by indicating that the excision sites flank the second selectable marker; 2) the recitation, "predetermined target sequence" in the last two lines of both claims be replaced with, --second selectable marker encoding nucleic acid--; and 3) in claim 13, it is suggested that the recitation, "at a predetermined target sequence such...following homologous recombination" in lines 6-9 of step b) be deleted.

In claim 13: the recitation, "excision sites" in line 7 of step b) renders the claim indefinite. It is unclear if these excision sites are the same as or different from the excision sites recited in line 3 of step b).

Further in claim 13: step f) renders the claim indefinite. The step indicates that the first DNA construct is to be introduced into plant cells from step e). However, step e) results in a plant. It is suggested that the recitation, --the plant of-- be inserted in line 2 of step f), after "from".

In claims 11 and 19: the claims are directed to a plant regenerated from the method of claim 1 or 13. However, different steps of the methods yield different plant cells. It is unclear which of these plant cells are regenerated into the plant of claims 11 and 19. It is suggested that

claims 11 and 19 be amended by inserting the following recitation after "from": --the plant cells selected in step e)-- (or step f), for claim 19).

In claims 12 and 20: the claims are directed to a system that comprises the constructs of claims 1 or 13. However, claims 1 and 13 are directed to methods, not constructs. It is suggested that the recitation, --the method of-- be inserted in line 2 of both claims, after "of".

In claim 15: the recitation, "associated" renders the claim indefinite. It is unclear exactly what is meant by the term in the context of the claim.

In claim 20: the recitation, "the construct of claim 13" renders the claim indefinite. However, claim 13 mentions two constructs. It is unclear which construct is being referred to.

In claims 21 and 22: it is unclear if the progeny plants comprise the first and second DNA constructs, minus the predetermined target sequence.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

4. Claims 1-22 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a site specific recombination method for removing predetermined nucleic acid sequences from a plastid genome, wherein a first nucleic acid construct is integrated into a plant cell nuclear genome and comprises a nucleic acid encoding a plastid targeting transit sequence operably linked to a nucleic acid encoding a site-specific recombinase, and a second DNA construct is introduced into the plastids, does not reasonably provide enablement for said method wherein the first nucleic acid construct does not comprise nucleic acid encoding a plastid

target sequence and nucleic acid not encoding a site-specific recombinase. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The claims are broadly drawn towards a site specific recombination method for removal of predetermined nucleic acid sequences from the plastid genome, said method comprising providing a) a first DNA construct comprising a promoter operably linked to an optional plastid targeting transit sequence, operably linked to a nucleic acid encoding any protein having any excision activity, said construct further comprising a selectable marker, b) a second DNA construct comprising flanking plastid targeting nucleic acid sequences which facilitate homologous recombination into the plastid genome, a second selectable marker and excision sites, and optionally a gene of interest c) introducing said second DNA construct into a plant cell, d) culturing said plant cell and selecting those expressing protein encoded by the second construct, e) introducing said first DNA construct into plant cells from step d), selecting those expressing proteins encoded by the first construct, wherein the excising activity acts on said excision sites, thereby excising the predetermined target sequence; or said method wherein a plant is regenerated from the plant cells of step d), and introducing the first DNA construct into those plant cells; or a plant regenerated from said method; or a site specific recombination system comprising said constructs.

The specification indicates that the Cre-lox site-specific recombination system was used in attempts to remove selective marker genes that had been introduced into genomes of plastids of plant cells. A plasmid for plastid genome transformation was constructed comprising the selectable marker, codA, operably linked to the Prrn promoter and TrbcL transcription

terminator. This chimeric codA gene was flanked by lox sites in the same orientation, which are recognized by the Cre recombinase. Another plasmid was also constructed, for transformation of the plant nuclear genome, which comprised a gene coding for the Cre recombinase, which also comprised sequences to target Cre to the plastid (pates 20-23). Tobacco plants were transformed with the construct comprising the codA gene to obtain transplastomic lines, which were purified to the homoplastomic state (pages 23-24). The plasmid containing the Cre gene was introduced into the nucleus of the transplastomic lines. The codA gene was removed in eight transgenic lines comprising the Cre gene. Two lines resulted in the unexpected deletion of both the coda gene and the adjacent aadA gene. The specification indicates that the latter deletion was the result of homologous recombination involving the Prrn promoter of the codA gene, and the Prrn promoter of an rRNA operon in the plastid genome (pages 26-27).

The methods of claims 1 and 13 indicate that the first nucleic acid construct comprises a promoter operably linked to a nucleic acid encoding a protein having excision activity. The nucleic acid of the first construct can broadly encode any protein having any type of excision activity. However, the specification does not enable the claimed methods with proteins having excision activity, other than those that are site-specific recombinases. The specification makes no suggestion of other proteins having other types of excision activity that can be used with the invention. In the absense of further guidance, undue experimentation would be required by one skilled in the art to determine how other types of excision activity can be used to with the claimed methods to remove predetermined nucleic acid sequences from the plastid genome of plant cells. See Genentech, Inc. v. Novo Nordisk, A/S, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997),

which teaches that "the specification, not the knowledge of one skilled in the art" must supply the enabling aspects of the invention.

Claims 1 and 13 also indicate that the operable linkage of the nucleic acid encoding the plastid targeting transit sequence to the nucleic acid encoding the protein having excision activity is optional. However, the specification does not enable practice of the claimed invention in the absence of this sequence. The specification admits that the plastid transit peptide is fused to Cre to ensure its targeting to the plastid (page 24). The method of the instant invention is also taught by Corneille et al. (Plant J., 2001, Vol. 27, pages 171-178). Corneille et al. teach that targeting of Cre to the plastids is critical to the method. Corneille et al. assert that Cre naturally carries a nuclear localization signal, that translational fusion with the Rubsico small subunit transit peptide yielded a protein with an N-terminal plastid targeting signal, and that when two subcellular localization signals are present, the N-terminal signal dominates the other (page 176). In the absence of further guidance, undue experimentation would be required by one skilled in the art to practice the claimed method to cause site-directed excision in plastids, when the Cre recombinase is not targeted to the plastid.

Further regarding claim 13: as written, the claim indicates in step b) that the second DNA construct comprises flanking plastid targeting nucleic acid sequences that facilitate homologous recombination into a plastid genome at a predetermined target sequence, such that excision sties flank the predetermined sequence following homologous recombination. However, the specification does not teach that such a sequence of events occur following plastid transformation. As discussed above, the specification teaches that the lox sites flank the selectable marker on this construct. There is no scenario taught in the specification in which lox

site flank a predetermined target sequence present in the plastid genome itself, following integration into the genome. Further, given that the predetermined target sequences on the construct facilitate homologous recombination into the genome, it is unclear how the lox sites can then flank the predetermined target sequence following integration. In the absence of further guidance, undue experimentation would be required by one skilled in the art to cause such a rearrangement of the excision sites of the second DNA construct following integration into the plastid genome.

Further regarding claims 6 and 17: the claims indicate that the excision sites of the claimed methods are LOX sequences and frt sequences. This encompasses one excision site being a LOX site while the other is a frt site. However, the same recombinase does not recognize both sites. Site-specific recombinases act at specific recombination, or excision, sites. Kilby et al. (Trends in Gen., 1993, Vol. 9, pages 413-421) teach that the Cre recombinase specifically recognizes LOX sites while the FLP recombinase specifically recognizes frt sites (page 413). Undue experimentation would be required by one skilled in the art to cause excision of the predetermined target sequence in the claimed method if the excision sites are not both recognized by the site-specific recombinase. In the absence of further guidance, unpredictability of the art and lack of guidance of the specification as discussed above, undue experimentation would be required by one skilled in the art to make and use the claimed invention.

5. Claims 1-22 are rejected.

Contact Information

Any inquiry concerning this communication from the Examiner should be directed to Ashwin Mehta, whose telephone number is 571-272-0803. The Examiner can normally be reached from 8:00 A.M to 5:30 P.M. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Amy Nelson, can be reached at 571-272-0804. The fax phone numbers for the organization where this application or proceeding is assigned are 571-273-8300. Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov.

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March 31, 2005

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